

University of Groningen

Agomelatine reverses the decrease in hippocampal cell survival induced by chronic mild stress

Dagyte, Girstaute; Crescente, Ilaria; Postema, Folkert; Seguin, Laure; Gabriel, Cecilia; Mocaer, Elisabeth; den Boer, Johan A.; Koolhaas, Jaap M.

Published in:
Behavioral Brain Research

DOI:
[10.1016/j.bbr.2010.11.045](https://doi.org/10.1016/j.bbr.2010.11.045)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2011

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Dagyte, G., Crescente, I., Postema, F., Seguin, L., Gabriel, C., Mocaer, E., den Boer, J. A., & Koolhaas, J. M. (2011). Agomelatine reverses the decrease in hippocampal cell survival induced by chronic mild stress. *Behavioral Brain Research*, 218(1), 121-128. <https://doi.org/10.1016/j.bbr.2010.11.045>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.



Research report

Agomelatine reverses the decrease in hippocampal cell survival induced by chronic mild stress

Girstautė Dagytė^{a,b,*}, Ilaria Crescente^{a,c}, Folkert Postema^{a,b}, Laure Seguin^d, Cecilia Gabriel^d, Elisabeth Mocaër^d, Johan A. Den Boer^b, Jaap M. Koolhaas^c

^a Department of Molecular Neurobiology, University of Groningen, The Netherlands

^b Department of Psychiatry, University of Groningen, The Netherlands

^c Department of Behavioral Physiology, University of Groningen, The Netherlands

^d Institut de Recherches Internationales Servier, Courbevoie, France

ARTICLE INFO

Article history:

Received 5 October 2010

Received in revised form

17 November 2010

Accepted 20 November 2010

Keywords:

Adult neurogenesis

Affective disorders

BrdU

DCX

Depression

Ki-67

ABSTRACT

The antidepressant agomelatine is a MT₁/MT₂ receptor agonist and 5-HT_{2C} antagonist. Its antidepressant activity is proposed to result from the synergy between these sets of receptors. Agomelatine-induced changes in the brain have been reported under basal conditions. Yet, little is known about its effects in the brain exposed to chronic stress as a risk factor for major depressive disorder. Recently, we described agomelatine-induced changes on neuronal activity and adult neurogenesis in the hippocampus of rats subjected to chronic footshock stress. In order to better characterize the actions of agomelatine in the stress-compromised brain, here we investigated its effects on hippocampal neurogenesis in the chronic mild stress (CMS) model. Adult male rats were subjected to various mild stressors for 5 weeks, and treated with agomelatine during the last 3 weeks of the stress period. The sucrose preference test was performed weekly to measure anhedonia, and the marble burying test was carried out at the end of the experiment to assess anxiety-like behavior. In our model, the CMS paradigm did not change sucrose preference; however, it increased marble burying behavior, indicating enhanced anxiety. Interestingly, this stress model differentially affected distinct stages of the neurogenesis process. Whereas CMS did not influence the rate of hippocampal cell proliferation, it significantly decreased the newborn cell survival and doublecortin expression in the dentate gyrus. Importantly, treatment with agomelatine completely normalized stress-affected cell survival and partly reversed reduced doublecortin expression. Taken together, these data show that agomelatine has beneficial effects on hippocampal neurogenesis in the CMS paradigm.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

Depression is a devastating illness and a major contributor to the global disease burden [1,2]. Treatment of depressive

disorder remains a challenge, despite of several classes of antidepressant drugs available. Currently, most of depressed patients are treated with monoaminergic compounds, such as selective serotonin reuptake inhibitors (SSRI), serotonin and norepinephrine reuptake inhibitors (SNRI), and norepinephrine reuptake inhibitors (NRI), which potentiate the brain's monoaminergic system and elevate the monoamine levels [3]. However, only about 50% of the depressed patients treated with these medications achieve complete remission [4,5]. Moreover, rather poor tolerability of currently used antidepressants and late onset of their therapeutic effects further increase the risk of unsuccessful treatment [6]. Thus, there is a need for novel and more efficient medications with fundamentally different mechanism of action.

Search for new targets in treatment of depression has led to the development of the first melatonergic antidepressant, agomelatine [7]. This novel drug acts as a potent agonist of melatonergic (MT₁/MT₂) receptors as well as an antagonist of the serotonergic (5-HT_{2C}) receptors [8,9]. The antidepressant properties of agome-

Abbreviations: 5-HT, serotonin; Ago, agomelatine; Akt, protein kinase B; BrdU, bromodeoxyuridine; BDNF, brain-derived neurotrophic factor; CMS, chronic mild stress; CREB, cyclic adenosine monophosphate responsive element binding protein; CTR, control group; DCX, doublecortin; ERK1/2, extracellular signal-regulated kinase 1/2; GCL, granular cell layer; GR, glucocorticoid receptor; GSK3β, glycogen synthase kinase 3β; HEC, hydroxyethylcellulose; ir, immunoreactivity; MR, mineralocorticoid receptor; MT, melatonin receptor; NRI, norepinephrine reuptake inhibitor; OD, optical density; SGZ, subgranular zone; SNRI, serotonin and norepinephrine reuptake inhibitor; SP, sucrose preference; SPT, sucrose preference test; SSRI, selective serotonin reuptake inhibitor; STR, stressed group; VEGF, vascular endothelial growth factor; Veh, vehicle.

* Corresponding author at: Department of Molecular Neurobiology, University of Groningen, P.O. Box 14, 9750 AA Haren, The Netherlands. Tel.: +31 50 363 2339; fax: +31 50 363 2331.

E-mail address: G.Dagyte@rug.nl (G. Dagytė).

latine have been demonstrated in both animal models [10–12] and in clinical studies [13–16]. The antidepressant efficacy of agomelatine includes beneficial effect on sleep quality, achieved via synergy between the melatonergic and 5-HT_{2C} receptors [17]. Current literature indicates that the therapeutic actions of agomelatine are mediated through different mechanisms as compared to SSRI and tricyclics [18,19]. Nevertheless, molecular correlates underlying the antidepressant action of agomelatine merit further investigation.

In an attempt to unravel the working mechanism of agomelatine, preclinical studies reported a variety of its mediated changes in the brain. In experiments with unchallenged laboratory animals, agomelatine was shown to enhance adult hippocampal neurogenesis, to increase expression of brain-derived neurotrophic factor (BDNF), and to activate several cellular signals, i.e. extracellular signal-regulated kinase 1/2 (ERK1/2), protein kinase B (Akt), and glycogen synthase kinase 3 β (GSK3 β) [20–22]. This information considerably advanced understanding of agomelatine's action in the brain. However, since effects of antidepressant drugs might differ in disturbed versus intact systems [6], the latter findings under basal conditions are not possible to directly extrapolate to the therapeutic action of agomelatine in depression. This gap may be partially bridged by applying an animal model approach, in order to elucidate effects of agomelatine under conditions that might predispose to mood disorders.

Animal models of chronic stress represent a valuable tool to investigate behavioral, endocrine and neurobiological changes underlying stress-related psychopathologies, such as major depression, and efficacy of antidepressant therapies [23,24]. The chronic mild stress (CMS) paradigm has been proposed as an animal model of depression [25]. In laboratory animals, this chronic stress procedure attempts to mimic some of the dysfunctions associated with human depressive disorder. As such, CMS was reported to induce anhedonia, which could be reversed by antidepressant treatment [10,26,27]. Also, CMS was demonstrated to reduce expression of BDNF and activation of cyclic adenosine monophosphate responsive element binding protein (CREB), both implicated in pathophysiology of depression and working mechanism of antidepressants [28–30]. Moreover, CMS was shown to suppress adult hippocampal neurogenesis, suggested to play a role in depressive disorder and action of antidepressant drugs [31–33]. Although impairments in hippocampal neurogenesis have not yet provided functional mechanism for the pathophysiology of depression, this phenomenon gained an enormous interest among researchers [34]. In particular, the notion that hippocampal neurogenesis may be oppositely regulated by stress and antidepressants gave rise to the possibility of using it as a read-out in testing the efficacy of novel treatments for major depressive disorder.

Altogether, this encouraged us to apply agomelatine treatment in chronically stressed animals, in order to explore its therapeutic actions and mechanisms behind them. Recently, we characterized agomelatine-induced changes in the brain of rats exposed to chronic footshock stress. In this model, treatment with agomelatine normalized stress-affected neuronal activity and promoted neurogenesis in the hippocampus [35]. In order to better characterize the action of agomelatine in the stress-compromised brain, here we investigated its effects on hippocampal neurogenesis in the CMS model. We asked whether this stress paradigm induced differential changes in distinct stages of the neurogenesis process (cell proliferation, survival and neuronal differentiation), and whether agomelatine treatment reversed them. In addition, we assessed effects of CMS and agomelatine on anhedonia and anxiety-like behavior by using sucrose preference and marble burying tests as respective behavioral paradigms.

2. Experimental procedures

2.1. Animals and housing

The experiments were performed using adult male Wistar rats (Harlan, Horst, The Netherlands) weighing 250–300 g at the beginning of the experiment. The animals were housed individually in a climate-controlled room with constant temperature ($21 \pm 1^\circ\text{C}$) and a 12 h light/12 h dark cycle (lights on at 05:00 h). Food and water was available *ad libitum*, except when food deprivation was applied as a stressor. The experiments were approved by the ethical committee for the use of experimental animals of the University of Groningen and carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.2. Experimental protocol

During a 2-week acclimatization period before the start of the experiment, all rats were handled, weighed and trained to consume 1% sucrose solution. Three habituation tests for sucrose were performed, followed by a baseline sucrose preference test (SPT). On the basis of their sucrose preference in the baseline SPT, animals were divided into two matched groups, home-cage control and chronic stress. Both groups were further subdivided into three subgroups, based on the drug treatment (untreated, treated with vehicle or agomelatine). Thus, six groups of rats were used for the present experiment: three groups of control rats, which were either untreated (CTR) or treated with vehicle (CTR-Veh) or agomelatine (CTR-Ago), and three groups of chronically stressed rats, which were also either untreated (STR) or treated with vehicle (STR-Veh) or agomelatine (STR-Ago). Each group consisted of 7–8 animals. Stress group rats were exposed to a 5-week CMS procedure, whereas control rats stayed undisturbed in a separate room. Drug treatment started on the third week of the experiment, and continued daily throughout the final 3 weeks of the 5-week experimental period. At the end of every week, the SPT was applied to all rats. In the last week of the experiment, the marble burying test was performed. At the end of the 5-week experiment, the home-cage control rats were sacrificed concomitantly with the stressed rats which were euthanized 4–6 h after the last stressor exposure and 18–20 h after the last treatment administration.

2.3. Chronic mild stress procedure

The CMS protocol was adapted from previous studies [10,36]. In the current experiment, the CMS procedure consisted of five different mild stressors, applied randomly every week for 5 weeks. These five stressors were: 16 h of food deprivation, 16 h of soiled cage (500 ml water in 250 g sawdust bedding), 16 h of tilted cage (45°), 12 h of predator sounds (high-pitch medium volume, resembling snake sounds) and 12 h of medium intensity of stroboscopic light flashes. These stressors were applied during the entire dark phase and first 4 h of the light phase, in case of the stressors with 16 h duration. Every experimental week consisted of 5 days with random stressors, and 2 days of no-stress.

2.4. Sucrose preference test

The sucrose preference test (SPT) was conducted as a measure for the anhedonic effect of CMS [36]. A two bottle preference test was used, where all animals got access to both water and a 1% sucrose solution for 24 h during a period of no-stress. In order to habituate animals to the sucrose solution, they were trained during the acclimatization period. Three habituation tests were performed, where animals had access to both water and sucrose solution for 24 h. This training period was followed by the baseline SPT, 1 day before the start of the CMS protocol. During the 5-week CMS procedure, SPT was performed at the end of each experimental week for 24 h during a period of no-stress. Bottles with water and sucrose solution were switched regularly to control for the place preference. The final SPT was performed 24 h before the last treatment administration. Sucrose preference (SP) was calculated as the percentage of sucrose solution ingested relative to the total amount of liquid consumed ($\text{SP} = [\text{sucrose solution, g/sucrose solution, g + water, g}] \times 100$).

2.5. Marble burying test

The marble burying test was used to measure an anxiety-induced behavioral response to environmental challenge. It was performed in the last week of the experiment, during the light phase of a stress-free period. The method was adapted from previous studies [37,38]. Briefly, four glass marbles (2.5 cm in diameter) were placed along a side-wall of each home-cage, and behavior of rats was observed during a 30-min test period. The following parameters were recorded: the number of rats showing burying behavior, and the number of buried marbles (at least two thirds of the surface covered with sawdust). Marble burying behavior reflected an active effort of a rat to hide the unfamiliar object in sawdust bedding, and therefore, it may indicate anxiety-like behavior [38].

2.6. Drug treatment

1% hydroxyethylcellulose (HEC) was used as a vehicle for agomelatine delivery. Agomelatine solution was prepared every day by dissolving agomelatine powder in 1% HEC at a concentration of 40 mg/ml. The choice of agomelatine dose was made on the basis of its activity at this concentration in animal models of depression and anxiety [10,39], and on neuroplasticity [20,21,35]. Rats were injected intraperitoneally either with agomelatine (40 mg/kg) or vehicle daily at 15:00 (2 h prior to the dark phase) for 21 days. Injections started on the third week of the 5-week experimental period. Agomelatine and HEC were provided by Servier (France).

2.7. BrdU labeling

In order to study survival of newly-born cells in the hippocampus during the experiment, rats received an injection of bromodeoxyuridine (BrdU; Sigma, St. Louis, MO, USA), a synthetic analogue of thymidine which is incorporated into newly synthesized DNA of replicating cells during the S phase of the cell cycle [40]. New cells were labeled 4 days before the start of the experiment with a single intraperitoneal injection (300 mg/kg). This specific time-point of BrdU labeling for studying cell survival was chosen since it was shown that labeled progenitor cells stop proliferating after 4 days [41]. Such an injection schedule was similarly applied in our laboratory previously [35,42,43].

2.8. Brain collection and immunohistochemistry

At the end of the experiment rats were anesthetized with sodium pentobarbital and transcardially perfused with heparinized saline followed by 4% paraformaldehyde solution in 0.1 M phosphate buffer. Brains were extracted and post-fixed in the same solution overnight at 4 °C and subsequently cryoprotected by immersion in a 30% buffered sucrose solution for up to 48 h. Coronal serial sections of 30 µm were cut using a cryostat and stored in 0.01 M phosphate-buffered saline with 0.1% sodium azide at 4 °C until immunohistochemistry was performed.

In order to assess effects of CMS and agomelatine on different aspects of hippocampal neurogenesis, immunohistochemistry for Ki-67, doublecortin (DCX), and BrdU was performed. Ki-67 is a nuclear protein which is expressed during all phases of the cell cycle, except G0 [44], which would therefore give an indication of cell proliferation on the last day of the experiment. DCX protein is expressed in newly-born cells from 1 day to about 4 weeks of age, and might therefore reveal sustained changes in the neurogenesis process and its modulation [45,46]. Finally, since a pool of newborn cells was labeled with an injection of BrdU 4 days prior to the start of the experiment, BrdU immunostaining would indicate changes in survival of new cells in the course of the experiment.

The immunostainings were performed on free-floating sections under continuous mild agitation. BrdU immunohistochemistry required extra steps for DNA denaturation. For this purpose, sections were exposed to 2× saline sodium citrate (2×SSC) containing 50% formamide for 30 min at 65 °C, followed by a rinse with 2×SSC, incubation with 2 M HCl for 30 min at 37 °C and a washing step with 0.1 M borate buffer. Brain sections for all three immunostainings were preincubated in 3% normal serum and 0.1% TritonX-100, and then incubated with one of the following antibodies for 60 h at 4 °C: primary mouse-anti-Ki-67 (1:200; Monosan, Uden, The Netherlands), goat-anti-DCX (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA), or rat-anti-BrdU (1:800; Serotec, Oxford, UK). Subsequently, sections were rinsed in 0.01 M Tris-buffered saline and incubated for 2 h at room temperature with secondary biotinylated goat-anti-mouse, rabbit-anti-goat or donkey-anti-rat antibody depending on the primary antibody host (1:500 for all secondary antibodies, Jackson ImmunoResearch Suffolk, UK). Then, avidin–biotin complex (1:500, Vector ABC kit, Vector Laboratories, Burlingame, CA, USA) was added for 2 h, after which the staining was visualized with 1 mg/ml diaminobenzidine and 0.003% H₂O₂. Thereafter, sections were rinsed, mounted on slides, dehydrated and coverslipped for microscopic analysis.

2.9. Quantification

All analyses were performed by an observer blind to the group assignment. Ki-67 and BrdU positive cells were counted in the subgranular zone (SGZ) and granular cell layer (GCL) of the dentate gyrus in every twelfth section of the hippocampus under 500× magnification. Immunopositive cells that were located one cell diameter away from SGZ were also included in the analysis. The number of immunopositive cells in each section was expressed relative to the length of the dentate gyrus in order to correct for variation in size of the hippocampus along the rostro-caudal axis of the brain. The length of the dentate gyrus was measured by delineating the entire SGZ in each hippocampus. DCX immunoreactivity (ir) in cell bodies and dendrites of the newly generated cells was measured by a previously described method [43,47]. Measurements were performed with a computerized system (Leica, Cambridge, UK). Images were taken using a Leica charged-coupled device digital camera mounted on a microscope (DMIRB, Leica, Cambridge, UK) at 100× magnification. In total, 7–8 sections per animal (Bregma –2.56 to –6.30, according to the rat brain atlas [48]) were analyzed by delineating a similarly sized area covering the entire width of the SGZ and GCL in each hippocampus. Optical density (OD) of DCX expression was measured in the dentate gyrus and corrected for non-specific background labeling,

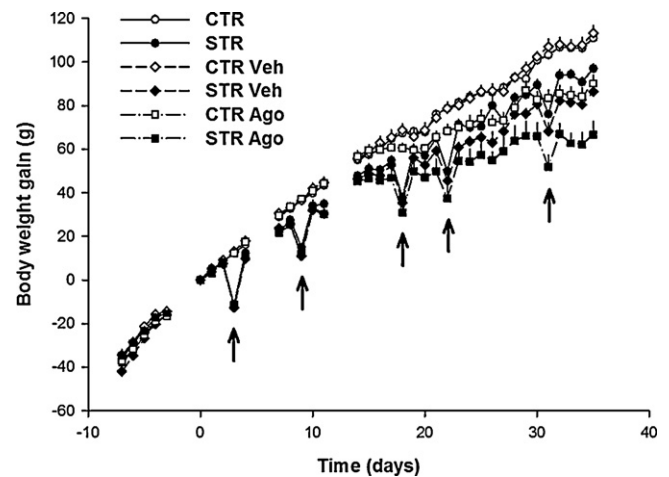


Fig. 1. Changes in body weight gain throughout the experiment. Both CMS and agomelatine treatment significantly reduced body weight gain. Arrows point at short-term body weight decreases due to the food deprivation stressor.

which was measured in the corpus callosum. The OD was expressed in arbitrary units corresponding to grey levels using a Quantimet 550 image analysis system (Leica, Cambridge, UK). Simultaneously, a second measure of the DCX immunostaining was taken, complementary to the DCX OD analysis. Within each delineated area covering the SGZ and GCL, the percentage of the total surface that was covered with DCX-positive cell bodies and dendrites was calculated, and presented as area coverage (%). In all three stainings, dorsal and ventral hippocampi were analyzed separately. Since similar changes in the dorsal and ventral parts of hippocampus were detected, these data were pooled and effects in the entire hippocampus were presented.

2.10. Statistics

Two-way ANOVA was used to analyze the effects of stress and drug treatment on hippocampal neurogenesis and marble burying. The body weight gain and sucrose preference data were analyzed by repeated measures ANOVA. When significant effects were found, post-hoc analysis was performed using the Fisher LSD test. The level of significance was set at $p < 0.05$.

3. Results

3.1. Body weight gain

Body weight was measured from day –7 to day 35 of the experiment. All rats grew at similar rates during the acclimatization period. However, CMS and agomelatine treatment significantly reduced body weight gain (repeated measures ANOVA, time × stress interaction: $F_{(30,1230)} = 32.85$, $p < 0.001$; time × drug interaction: $F_{(60,1230)} = 15.79$, $p < 0.001$; Fig. 1).

3.2. Sucrose preference test

Preference for 1% sucrose solution over water was measured before the start of the CMS procedure (baseline) and weekly during the 5-week experimental period. Repeated measures ANOVA did not reveal any significant differences in sucrose preference among the groups throughout the experiment ($p > 0.05$; Fig. 2).

3.3. Marble burying test

The number of buried marbles by each animal was recorded during a 30-min test period. Fig. 3A depicts the average number of buried marbles in each experimental group. Individual variation associated with this behavioral measure is illustrated in Fig. 3B. The data points correspond to individual animals, and show the number of their buried marbles. Two-way ANOVA revealed a significant stress-induced increase in burying behavior ($F_{(1,41)} = 5.18$, $p = 0.03$, when the number of animals showing burying behavior was ana-

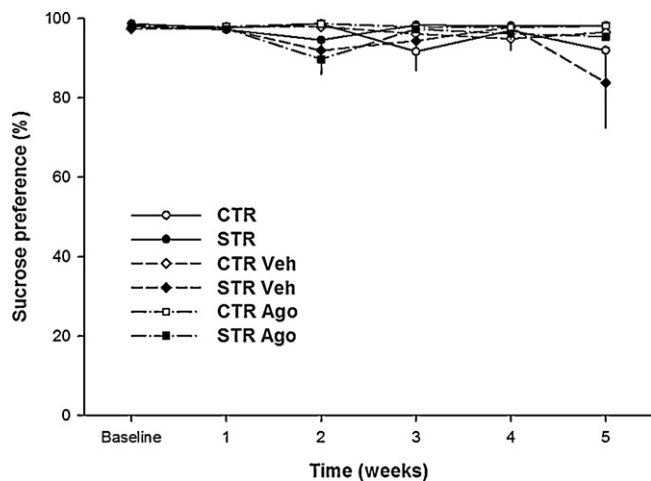


Fig. 2. Effects of CMS and agomelatine treatment on sucrose preference. A preference for 1% sucrose solution over water was measured before the start of the CMS procedure (baseline) and weekly during the 5-week experimental period. CMS did not induce significant changes in sucrose preference among the groups throughout the experiment.

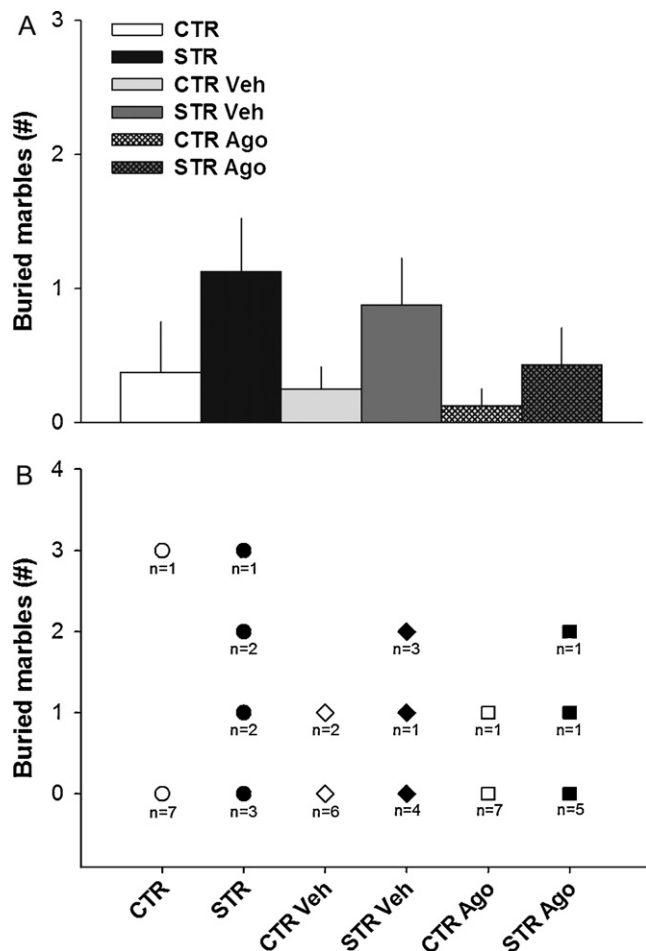


Fig. 3. Effects of CMS and agomelatine treatment on marble burying. The graph depicts the average number of buried marbles in each experimental group (A). This measure was associated with a high individual variation, and not all animals showed burying behavior (B). The data points correspond to individual rats, and reveal the number of their buried marbles. Some data points represent more than one animal; their *n* is indicated below each point. CMS induced a significant increase in burying behavior (both in the number of rats showing burying behavior and in the number of their buried marbles). Although agomelatine treatment seemed to reduce burying behavior, its effects did not reach statistical significance, $p < 0.05$ (overall stress effect).

lyzed; $F_{(1,41)} = 5.06$, $p = 0.03$, when the number of buried marbles across the groups was compared). Agomelatine treatment reduced burying behavior (only 29% of stressed agomelatine-treated rats buried marbles as compared to 63% of stressed non-injected and 50% of stressed vehicle-treated animals), but this effect did not reach statistical significance.

3.4. Hippocampal neurogenesis

Fig. 4 shows representative examples of Ki-67 (A), BrdU (B) and DCX (C) immunostainings in the hippocampal dentate gyrus. Changes in different stages of hippocampal neurogenesis after CMS and agomelatine treatment are depicted in Fig. 5.

Hippocampal cell proliferation was assessed by Ki-67 immunohistochemistry (Fig. 4A). There were no significant differences in the number of proliferating cells among the groups ($p > 0.05$; Fig. 5A).

Hippocampal cell survival was assessed by BrdU immunohistochemistry (Fig. 4B). Two-way ANOVA revealed significant stress and drug effects ($F_{(1,41)} = 9.77$, $p = 0.003$ and $F_{(2,41)} = 4.20$, $p = 0.02$, respectively; Fig. 5B). Post-hoc Fisher LSD test showed that CMS significantly reduced the number of BrdU-positive cells in both untreated and vehicle treated animals (CTR vs. STR: $p = 0.03$; CTR-Veh vs. STR-Veh: $p = 0.01$). Agomelatine reversed this stress-induced effect (STR-Veh vs. STR-Ago: $p = 0.02$), whereas it did not influence the rate of cell survival in control animals (CTR-Veh vs. CTR-Ago: $p > 0.05$).

Effects of CMS and agomelatine on newly-born hippocampal neuron maturation were assessed by DCX labeling (Fig. 4C). First, we quantified the OD of DCX expression (Fig. 5C). Two-way ANOVA revealed a significant stress effect and a stress \times drug interaction ($F_{(1,41)} = 12.38$, $p = 0.001$ and $F_{(2,41)} = 11.41$, $p < 0.001$, respectively). Post-hoc Fisher LSD test showed that both CMS and agomelatine decreased the OD of DCX expression in the dentate gyrus (CTR vs. STR: $p = 0.03$; CTR-Veh vs. STR-Veh: $p < 0.001$; CTR-Veh vs. CTR-Ago: $p < 0.001$). In stressed animals, agomelatine significantly reversed the decrease in DCX expression (STR-Veh vs. STR-Ago: $p = 0.04$), but did not restore it to the control level (CTR-Veh vs. STR-Ago: $p = 0.004$). Additionally, we analyzed the percentage of area covered with DCX-positive cells (Fig. 5D). Two-way ANOVA revealed a significant stress \times drug interaction ($F_{(2,41)} = 6.89$, $p = 0.003$). Post-hoc Fisher LSD test showed that both CMS and agomelatine decreased the percentage of dentate gyrus area covered with DCX-positive cells (CTR vs. STR: $p = 0.008$; CTR-Veh vs. STR-Veh: $p = 0.015$; CTR-Veh vs. CTR-Ago: $p = 0.002$). Agomelatine increased DCX area coverage in stressed animals, but this effect did not reach statistical significance ($p > 0.05$).

4. Discussion

The present study investigated action of agomelatine in the rat CMS model. Our data show that this stress paradigm differentially affected distinct stages of the adult neurogenesis process. Whereas CMS did not influence the rate of hippocampal cell proliferation, it significantly decreased the newborn cell survival and doublecortin expression in the dentate gyrus. Notably, treatment with agomelatine completely normalized stress-affected cell survival and partly reversed the reduced doublecortin expression. Altogether, these data show that agomelatine has beneficial effects on hippocampal neurogenesis in the stress-compromised brain.

Exposure to CMS led to a significant decrease in body weight gain. Similar effects on growth were previously reported in male rats subjected to various chronic stress models [43,49,50]. In the CMS model, reduction in growth may depend on a combination of specific stressors, since both decrease [51–53] and no change

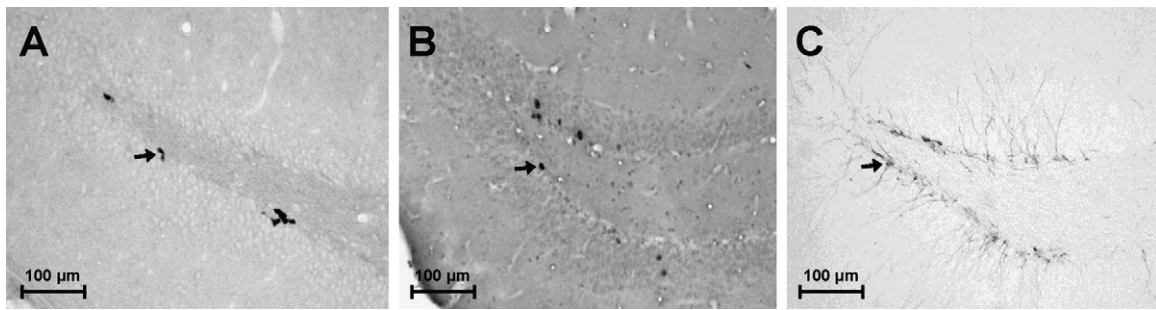


Fig. 4. Representative photomicrographs of Ki-67 (A), BrdU (B) and DCX (C) immunostainings in the hippocampal dentate gyrus. Arrows point at Ki-67 (A), BrdU (B) and DCX (C) labeled cells.

in body weight [28,54] were found in this stress paradigm. In our experiment, the food deprivation stressor had obvious effects on growth; however, its induced weight loss was short-lasting and fully recovered on the following day. Thus, the overall reduction in growth in the current CMS model cannot be attributed to a single food deprivation stressor, but rather depends on a synergistic effect of multiple stressors. The 3-week treatment with agomelatine also led to a significant decrease in body weight gain, as observed in our previous study [35]. This reduction in growth is most likely mediated through agomelatine's action on melatonergic receptors, as melatonin was reported to decrease body weight in rodents [55]. In contrast to the growth reduction in rats, agomelatine treatment does not change body weight in humans [56,57].

Our results reveal that exposure to the CMS paradigm did not change the rate of hippocampal cell proliferation. Nevertheless, it significantly decreased the survival of hippocampal cells, born before the stress period. Furthermore, CMS was associated with a reduction in the expression of DCX, a protein present mainly in the newborn immature neurons. It is interesting to note that distinct aspects of the neurogenesis process might be differentially influenced by stressful stimuli. Our data confirm a previously observed discrepancy between stress effects on hippocampal cell proliferation and survival in the CMS model [58]. A similar dissociation between different stages of neurogenesis was also found in a related paradigm of chronic unpredictable stress [59]. These results suggest that the CMS paradigm influences a part, but not the entire process of hippocampal neurogenesis. Such differential effects associated with CMS may hint to the role of this form of stress in regulation of adult neurogenesis in the hippocampus. Sustained glucocorticoid exposure is considered as one of the mediators of stress-induced changes in neurogenesis [24,60]. A degree of the hypothalamic–pituitary–adrenal (HPA) axis activation in the CMS model varies across different studies. Whereas some researchers report mild to moderate increases in plasma corticosterone concentrations after exposure to CMS [52–54,61], others do not find lasting changes in basal corticosterone levels [58,62], as also observed in our laboratory (unpublished results). The action of corticosterone is mediated through two types of receptors, the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR). Both of these receptors are barely found on hippocampal progenitors, but their expression increases dynamically while newborn cells progress through different stages of the neurogenesis process to develop into mature neurons [63,64]. Altogether, this points to a limited role of corticosteroids in regulating adult neurogenesis in the CMS model. Furthermore, these observations suggest that a variable sensitivity of the newborn cells to changes in the local glucocorticoid environment might influence stress effects on different aspects of the neurogenesis process [65,66]. As mentioned above, proliferating cells have low GR and MR levels that may contribute to the lack of CMS effects on the rate of cell proliferation. In contrast, the survival of older hippocampal cells, expressing higher GR and

MR levels, may be more severely influenced by stress. In addition, many other factors, including various proteins, neurotransmitters and hormones, are suggested to regulate the proliferation and survival of newborn cells, and have differential effects on these two processes [67,68]. Growth factors, especially, vascular endothelial growth factor (VEGF) and BDNF, seem to play a crucial role in regulating survival and differentiation of newly generated cells [69,70]. In line with this notion, reduced neurotrophin expression has been repeatedly reported in the CMS model [28,29,61,71]. In sum, adult hippocampal neurogenesis, and cell proliferation in particular, is a highly dynamic process [43,72]. Hence, the future research should consider the temporal dynamics of the stress response and time-dependent effects of various stress mediators when designing new studies of this kind [73,74].

Importantly, agomelatine treatment completely reversed the CMS-induced decrease in hippocampal cell survival. This finding confirms the previous observations on actions of agomelatine in the stress-compromised brain [35,75] as well as in a transgenic animal model of depression [19]. Moreover, our data show that this antidepressant partly reversed stress-induced reduction in the DCX labeling in the dentate gyrus. Agomelatine treatment in stressed animals significantly increased the OD of DCX expression; however, its effects on the second measure of this analysis, the area covered with DCX-positive cells, did not reach statistical significance. Such discrepancy might indicate that distinct dimensions measured by two methods differ in their magnitude. The OD reveals the intensity of the staining, and thus, reflects the amount of expressed protein. Previously, we showed that changes in the OD of DCX labeling correlate with alterations in the number of immature DCX-positive neurons [43]. We also acknowledged the differential influence of neurogenic regulators, such as stress and antidepressants, on DCX-positive cells [35]. Earlier studies demonstrated that DCX protein is expressed dynamically by heterogeneous cell populations, ranging from proliferating progenitors to immature yet post-mitotic neurons [76–78]. A possibility that stress and antidepressant treatment differentially regulate the distinct populations of DCX cells was also confirmed in the CMS model [32]. According to the proposed hypothesis, chronic stress might preferentially affect DCX-expressing post-mitotic neurons, whereas antidepressant drugs might promote the proliferating DCX-positive progenitors and accelerate their progression towards mature neurons [32,79–81]. Our DCX data show that CMS reduced both the amount of this protein as well as the relative number of cell bodies and dendrites that express it. It is feasible that stress downregulates the expression of DCX by decreasing the number of immature neurons. On the other hand, agomelatine also reduced both of these measures. Previous studies revealed that this antidepressant speeds up maturation of newborn neurons [21], resulting in lower number of DCX-positive cells [20], and thereby, reduced expression of DCX protein [35]. Interestingly, the present data show that agomelatine significantly, albeit partly, counteracted the

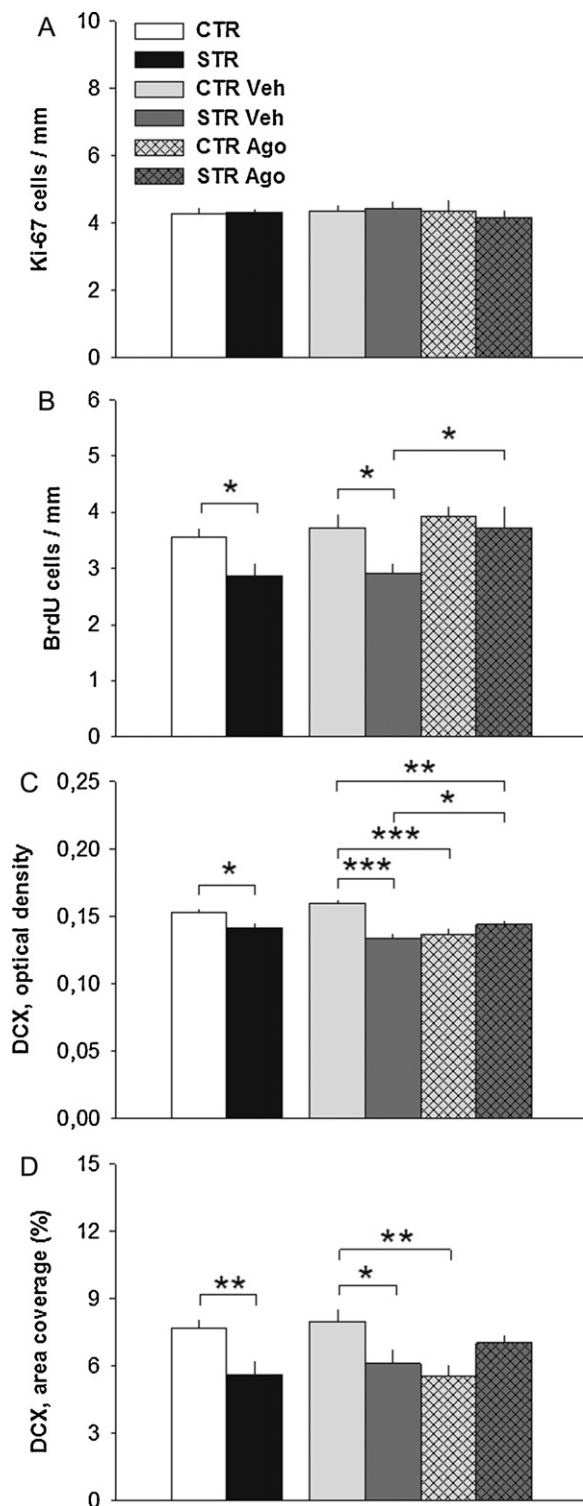


Fig. 5. Effects of CMS and agomelatine treatment on adult hippocampal neurogenesis. Neither CMS nor agomelatine influenced hippocampal cell proliferation (A). Whereas CMS decreased hippocampal cell survival, agomelatine completely reversed this stress effect (B). Both CMS and agomelatine treatment reduced OD of the DCX expression in the dentate gyrus, whereas a combination of stress and drug treatment partly rescued this effect (C). Accordingly, both CMS and agomelatine decreased the percentage of dentate gyrus area covered with the DCX-positive neurons (D). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

stress-induced decrease in DCX reactivity but did not significantly change the relative number of cells that express this protein. The effect of agomelatine on stress-altered DCX immunoreactivity was rather mild. Therefore, it might have not manifested as significant

change in the measurement of area of coverage, associated with higher individual variation.

Notably, our data show that agomelatine treatment did not influence hippocampal cell proliferation or survival in non-stressed rats. These findings are in line with our previous observations [35], but contrast from earlier reports [20,21]. Discrepant effects among studies may be due to subtle changes in the experimental protocols as discussed in more detail previously [35].

The neurobiological effects achieved in the CMS model granted valuable information on the action of agomelatine in the stress-compromised brain. Strikingly, however, CMS did not induce anhedonia as measured by preference for 1% sucrose solution over water. In fact, rats showed a very high preference for sucrose which did not diminish during a 5-week stress period. Both control and stressed animals drank an excessive amount of sweetened solution as compared to their daily water intake. Such elevated sucrose consumption indicates that rats liked/wanted it highly. Notably, different neural systems underlie the conditions of liking and wanting of pleasurable stimuli, suggesting a possible dissociation between these processes [82]. Although usually a brain likes the rewards that it wants, yet, sometimes it may just want them [83]. In view of this, the excess intake of sucrose solution may indicate a strong wanting component of reward, not necessarily accompanied by a liking element. The measurement of sucrose consumption over water, thus, might not adequately reflect the hedonic behavior. Consequently, data on sucrose intake and preference in the CMS studies should be interpreted with caution. In some cases, for instance, the so called anhedonic state is a result of increased sucrose consumption by control animals, without a change in sucrose intake by their stressed counterparts [28]. Altogether, there is a great deal of controversy in the methods used for determination of anhedonia, and in their measured effects in CMS studies. Despite numerous reports on stress-induced changes in either sucrose preference or intake [10,27,29,33,51,54,61,84,85], literature also reveals many contrasting results [71,86–90]. Such controversy is suggested to stem from differences in experimental designs and animal strains used; however, a solid explanation for discrepant findings is still lacking [86,89,91].

Interestingly, our CMS paradigm induced changes in marble burying behavior. Both the number of rats showing this behavior as well as the number of their buried marbles was increased in the groups subjected to CMS. The marble burying test is suggested to measure anxiety-like and impulsive behavior, and is proposed to detect potential antidepressant drugs; yet, the functional interpretation of marble burying remains uncertain [92–94]. Nevertheless, our findings reveal that exposure to CMS influenced the animals' response to a novel object in the cage. Increased burying behavior might indicate stress-heightened anxiety [95]. Although agomelatine had no statistically significant effects in the marble burying test, it did show a tendency to reduce the burying behavior. This observation may reflect the anxiolytic action of agomelatine, previously demonstrated in various animal models [39,96], including the marble burying test (unpublished results).

In summary, the present study used the CMS model in order to elucidate effects of stress and agomelatine treatment on adult hippocampal neurogenesis. Interestingly, this stress paradigm differentially affected distinct aspects of the neurogenesis process: whereas CMS did not alter the rate of cell proliferation, it decreased the newborn cell survival and DCX expression in the dentate gyrus. Importantly, treatment with agomelatine interfered with these stress-associated changes in the brain. This antidepressant completely normalized stress-affected cell survival and partly reversed reduced DCX expression. Taken together, these findings reveal the ability of agomelatine to counteract stress-induced changes in hippocampal neurogenesis, and confirm its previously reported actions in the stress-compromised brain.

Acknowledgments

This study was partly supported by a grant from Servier (France). We would like to thank Prof. Eddy Van der Zee for his helpful comments and Jan Keijser for technical assistance with the Quantimet image analysis system.

References

- [1] Greden JF. The burden of recurrent depression: causes, consequences, and future prospects. *J Clin Psychiatry* 2001;62(Suppl. 22):5–9.
- [2] Greenberg PE, Birnbaum HG. The economic burden of depression in the US: societal and patient perspectives. *Expert Opin Pharmacother* 2005;6:369–76.
- [3] Wong ML, Licinio J. Research and treatment approaches to depression. *Nat Rev Neurosci* 2001;2:343–51.
- [4] Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM. Neurobiology of depression. *Neuron* 2002;34:13–25.
- [5] Fagioli A, Kupfer DJ. Is treatment-resistant depression a unique subtype of depression? *Biol Psychiatry* 2003;53:640–8.
- [6] Berton O, Nestler EJ. New approaches to antidepressant drug discovery: beyond monoamines. *Nat Rev Neurosci* 2006;7:137–51.
- [7] de Bodinat C, Guardiola-Lemaitre B, Mocaer E, Renard P, Munoz C, Millan MJ. Agomelatine, the first melatonergic antidepressant: discovery, characterization and development. *Nat Rev Drug Discov* 2010;9:628–42.
- [8] Millan MJ, Gobert A, Lejeune F, Dekeyne A, Newman-Tancredi A, Pasteau V, et al. The novel melatonin agonist agomelatine (S20098) is an antagonist at 5-hydroxytryptamine_{2C} receptors, blockade of which enhances the activity of frontocortical dopaminergic and adrenergic pathways. *J Pharmacol Exp Ther* 2003;306:954–64.
- [9] Audinot V, Mailliet F, Lahaye-Brasseur C, Bonnaud A, Le Gall A, Amosse C, et al. New selective ligands of human cloned melatonin MT₁ and MT₂ receptors. *Naunyn-Schmiedeberg's Arch Pharmacol* 2003;367:553–61.
- [10] Papp M, Gruca P, Boyer PA, Mocaer E. Effect of agomelatine in the chronic mild stress model of depression in the rat. *Neuropsychopharmacology* 2003;28:694–703.
- [11] Bertaina-Anglade V, la Rochelle CD, Boyer PA, Mocaer E. Antidepressant-like effects of agomelatine (S 20098) in the learned helplessness model. *Behav Pharmacol* 2006;17:703–13.
- [12] Bourin M, Mocaer E, Porsolt R. Antidepressant-like activity of S 20098 (agomelatine) in the forced swimming test in rodents: involvement of melatonin and serotonin receptors. *J Psychiatry Neurosci* 2004;29:126–33.
- [13] Kennedy SH, Emsley R. Placebo-controlled trial of agomelatine in the treatment of major depressive disorder. *Eur Neuropsychopharmacol* 2006;16:93–100.
- [14] Montgomery SA. Why do we need new and better antidepressants? *Int Clin Psychopharmacol* 2006;21(Suppl. 1):S1–10.
- [15] Olie JP, Kasper S. Efficacy of agomelatine, a MT₁/MT₂ receptor agonist with 5-HT_{2C} antagonistic properties, in major depressive disorder. *Int J Neuropsychopharmacol* 2007;10:661–73.
- [16] Goodwin GM, Emsley R, Rembry S, Rouillon F. Agomelatine prevents relapse in patients with major depressive disorder without evidence of a discontinuation syndrome: a 24-week randomized, double-blind, placebo-controlled trial. *J Clin Psychiatry* 2009;70:1128–37.
- [17] Popoli M. Agomelatine: innovative pharmacological approach in depression. *CNS Drugs* 2009;23(Suppl. 2):27–34.
- [18] Hanou N, Mocaer E, Boyer PA, Hamon M, Lanfumey L. Differential effects of the novel antidepressant agomelatine (S 20098) versus fluoxetine on 5-HT_{1A} receptors in the rat brain. *Neuropharmacology* 2004;47:515–26.
- [19] Paizanis E, Renoit T, Lelievre V, Saurini F, Melfort M, Gabriel C, et al. Behavioural and neuroplastic effects of the new-generation antidepressant agomelatine compared to fluoxetine in glucocorticoid receptor-impaired mice. *Int J Neuropsychopharmacol* 2010;13:759–74.
- [20] Banas M, Soumier A, Hery M, Mocaer E, Daszuta A, Agomelatine. a new antidepressant, induces regional changes in hippocampal neurogenesis. *Biol Psychiatry* 2006;59:1087–96.
- [21] Soumier A, Banas M, Lortet S, Masmejean F, Bernard N, Kerkerian-Le-Goff L, et al. Mechanisms contributing to the phase-dependent regulation of neurogenesis by the novel antidepressant, agomelatine, in the adult rat hippocampus. *Neuropsychopharmacology* 2009;34:2390–403.
- [22] Molteni R, Calabrese F, Pisoni S, Gabriel C, Mocaer E, Racagni G, et al. Synergistic mechanisms in the modulation of the neurotrophin BDNF in the rat prefrontal cortex following acute agomelatine administration. *World J Biol Psychiatry* 2010;11:148–53.
- [23] Nestler EJ, Gould E, Manji H, Buncan M, Duman RS, Greshenfeld HK, et al. Preclinical models: status of basic research in depression. *Biol Psychiatry* 2002;52:503–28.
- [24] Fuchs E, Flugge G, Ohl F, Lucassen P, Vollmann-Honsdorf GK, Michaelis T. Psychosocial stress, glucocorticoids, and structural alterations in the tree shrew hippocampus. *Physiol Behav* 2001;73:285–91.
- [25] Willner P, Muscat R, Papp M. Chronic mild stress-induced anhedonia: a realistic animal model of depression. *Neurosci Biobehav Rev* 1992;16:525–34.
- [26] Willner P. Validity, reliability and utility of the chronic mild stress model of depression: a 10-year review and evaluation. *Psychopharmacology (Berl)* 1997;134:319–29.
- [27] Bessa JM, Ferreira D, Melo I, Marques F, Cerqueira JJ, Palha JA, et al. The mood-improving actions of antidepressants do not depend on neurogenesis but are associated with neuronal remodeling. *Mol Psychiatry* 2009;14:764–73, 39.
- [28] Gronli J, Bramham C, Murison R, Kanhema T, Fiske E, Bjorvatn B, et al. Chronic mild stress inhibits BDNF protein expression and CREB activation in the dentate gyrus but not in the hippocampus proper. *Pharmacol Biochem Behav* 2006;85:842–9.
- [29] Hu Y, Liao HB, Dai-Hong G, Liu P, Wang YY, Rahman K. Antidepressant-like effects of 3,6'-disinapoyl sucrose on hippocampal neuronal plasticity and neurotrophic signal pathway in chronically mild stressed rats. *Neurochem Int* 2010;56:461–5.
- [30] Castren E, Voikar V, Rantamaki T. Role of neurotrophic factors in depression. *Curr Opin Pharmacol* 2007;7:18–21.
- [31] Krishnan V, Nestler EJ. The molecular neurobiology of depression. *Nature* 2008;455:894–902.
- [32] Jayatissa MN, Bisgaard CF, West MJ, Wiborg O. The number of granule cells in rat hippocampus is reduced after chronic mild stress and re-established after chronic escitalopram treatment. *Neuropharmacology* 2008;54:530–41.
- [33] Elizalde N, Garcia-Garcia AL, Totterdell S, Gendive N, Venzala E, Ramirez MJ, et al. Sustained stress-induced changes in mice as a model for chronic depression. *Psychopharmacology (Berl)* 2010;210:393–406.
- [34] Dagyte G, Den Boer JA, Trentani A. The cholinergic system and depression. *Behav Brain Res* 2010. In press.
- [35] Dagyte G, Trentani A, Postema F, Luiten PG, Den Boer JA, Gabriel C, et al. The novel antidepressant agomelatine normalizes hippocampal neuronal activity and promotes neurogenesis in chronically stressed rats. *CNS Neurosci Ther* 2010;16:195–207.
- [36] Willner P, Towell A, Sampson D, Sophokleous S, Muscat R. Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. *Psychopharmacology (Berl)* 1987;93:358–64.
- [37] Pandey DK, Yadav SK, Mahesh R, Rajkumar R. Depression-like and anxiety-like behavioural aftermaths of impact accelerated traumatic brain injury in rats: a model of comorbid depression and anxiety? *Behav Brain Res* 2009;205:436–42.
- [38] Ho YJ, Eichendorff J, Schwarting RK. Individual response profiles of male Wistar rats in animal models for anxiety and depression. *Behav Brain Res* 2002;136:1–12.
- [39] Millan MJ, Brocco M, Gobert A, Dekeyne A. Anxiolytic properties of agomelatine, an antidepressant with melatonergic and serotonergic properties: role of 5-HT_{2C} receptor blockade. *Psychopharmacology (Berl)* 2005;177:448–58.
- [40] Christie BR, Cameron HA. Neurogenesis in the adult hippocampus. *Hippocampus* 2006;16:199–207.
- [41] Dayer AG, Ford AA, Cleaver KM, Yassaee M, Cameron HA. Short-term and long-term survival of new neurons in the rat dentate gyrus. *J Comp Neurol* 2003;460:563–72.
- [42] Van der Borgh K, Meerlo P, Luiten PG, Eggen BJ, Van der Zee EA. Effects of active shock avoidance learning on hippocampal neurogenesis and plasma levels of corticosterone. *Behav Brain Res* 2005;157:23–30.
- [43] Dagyte G, Van der Zee EA, Postema F, Luiten PG, Den Boer JA, Trentani A, et al. Chronic but not acute foot-shock stress leads to temporary suppression of cell proliferation in rat hippocampus. *Neuroscience* 2009;162:904–13.
- [44] Scholzen T, Gerdes J. The Ki-67 protein: from the known and the unknown. *J Cell Physiol* 2000;182:311–22.
- [45] Rao MS, Shetty AK. Efficacy of doublecortin as a marker to analyse the absolute number and dendritic growth of newly generated neurons in the adult dentate gyrus. *Eur J Neurosci* 2004;19:234–46.
- [46] Brown JP, Couillard-Despres S, Cooper-Kuhn CM, Winkler J, Aigner L, Kuhn HG. Transient expression of doublecortin during adult neurogenesis. *J Comp Neurol* 2003;467:1–10.
- [47] Van der Borgh K, Havekes R, Bos T, Eggen BJ, Van der Zee EA. Exercise improves memory acquisition and retrieval in the Y-maze task: relationship with hippocampal neurogenesis. *Behav Neurosci* 2007;121:324–34.
- [48] Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 6th ed. Academic Press; 2007.
- [49] Karst H, Joels M. Effect of chronic stress on synaptic currents in rat hippocampal dentate gyrus neurons. *J Neurophysiol* 2003;89:625–33.
- [50] Rygula R, Abumaria N, Flugge G, Fuchs E, Ruther E, Havemann-Reinecke U. Anhedonia and motivational deficits in rats: impact of chronic social stress. *Behav Brain Res* 2005;162:127–34.
- [51] Jayatissa MN, Henningsen K, West MJ, Wiborg O. Decreased cell proliferation in the dentate gyrus does not associate with development of anhedonic-like symptoms in rats. *Brain Res* 2009;1290:133–41.
- [52] Bielajew C, Konkole AT, Merali Z. The effects of chronic mild stress on male Sprague-Dawley and Long Evans rats: I. Biochemical and physiological analyses. *Behav Brain Res* 2002;136:583–92.
- [53] Ushijima K, Morikawa T, To H, Higuchi S, Ohdo S. Chronobiological disturbances with hyperthermia and hypercortisolism induced by chronic mild stress in rats. *Behav Brain Res* 2006;173:326–30.
- [54] Grippo AJ, Francis J, Beltz TG, Felder RB, Johnson AK. Neuroendocrine and cytokine profile of chronic mild stress-induced anhedonia. *Physiol Behav* 2005;84:697–706.
- [55] Wolden-Hanson T, Mitton DR, McCants RL, Yellon SM, Wilkinson CW, Matsumoto AM, et al. Daily melatonin administration to middle-aged male rats suppresses body weight, intraabdominal adiposity, and plasma leptin and insulin independent of food intake and total body fat. *Endocrinology* 2000;141:487–97.

- [56] Kasper S, Hamon M. Beyond the monoaminergic hypothesis: agomelatine, a new antidepressant with an innovative mechanism of action. *World J Biol Psychiatry* 2009;10:117–26.
- [57] Rouillon F. Efficacy and tolerance profile of agomelatine and practical use in depressed patients. *Int Clin Psychopharmacol* 2006;21(Suppl. 1):S31–5.
- [58] Lee KJ, Kim SJ, Kim SW, Choi SH, Shin YC, Park SH, et al. Chronic mild stress decreases survival, but not proliferation, of new-born cells in adult rat hippocampus. *Exp Mol Med* 2006;38:44–54.
- [59] Oomen CA, Mayer JL, de Kloet ER, Joels M, Lucassen PJ. Brief treatment with the glucocorticoid receptor antagonist mifepristone normalizes the reduction in neurogenesis after chronic stress. *Eur J Neurosci* 2007;26:3395–401.
- [60] de Kloet ER, Joels M, Holsboer F. Stress and the brain: from adaptation to disease. *Nat Rev Neurosci* 2005;6:463–75.
- [61] Toth E, Gersner R, Wilf-Yarkoni A, Raizel H, Dar DE, Richter-Levin G, et al. Age-dependent effects of chronic stress on brain plasticity and depressive behavior. *J Neurochem* 2008;107:522–32.
- [62] Stout SC, Mortas P, Owens MJ, Nemeroff CB, Moreau J. Increased corticotropin-releasing factor concentrations in the bed nucleus of the stria terminalis of anhedonic rats. *Eur J Pharmacol* 2000;401:39–46.
- [63] Cameron HA, Woolley CS, Gould E. Adrenal steroid receptor immunoreactivity in cells born in the adult rat dentate gyrus. *Brain Res* 1993;611:342–6.
- [64] Garcia A, Steiner B, Kronenberg G, Bick-Sander A, Kempermann G. Age-dependent expression of glucocorticoid- and mineralocorticoid receptors on neural precursor cell populations in the adult murine hippocampus. *Aging Cell* 2004;3:363–71.
- [65] Wong EY, Herbert J. The corticoid environment: a determining factor for neural progenitors' survival in the adult hippocampus. *Eur J Neurosci* 2004;20:2491–8.
- [66] Wong EY, Herbert J. Roles of mineralocorticoid and glucocorticoid receptors in the regulation of progenitor proliferation in the adult hippocampus. *Eur J Neurosci* 2005;22:785–92.
- [67] Hagg T. Molecular regulation of adult CNS neurogenesis: an integrated view. *Trends Neurosci* 2005;28:589–95.
- [68] Balu DT, Lucki I. Adult hippocampal neurogenesis: regulation, functional implications, and contribution to disease pathology. *Neurosci Biobehav Rev* 2009;33:232–52.
- [69] Mattson MP, Maudsley S, Martin B. BDNF and 5-HT: a dynamic duo in age-related neuronal plasticity and neurodegenerative disorders. *Trends Neurosci* 2004;27:589–94.
- [70] Cao L, Jiao X, Zuzga DS, Liu Y, Fong DM, Young D, et al. VEGF links hippocampal activity with neurogenesis, learning and memory. *Nat Genet* 2004;36:827–35.
- [71] Bergstrom A, Jayatissa MN, Mork A, Wiborg O. Stress sensitivity and resilience in the chronic mild stress rat model of depression; an in situ hybridization study. *Brain Res* 2008;1196:41–52.
- [72] Ma DK, Kim WR, Ming GL, Song H. Activity-dependent extrinsic regulation of adult olfactory bulb and hippocampal neurogenesis. *Ann N Y Acad Sci* 2009;1170:664–73.
- [73] Buwalda B, Felszeghy K, Horvath KM, Nyakas C, de Boer SF, Bohus B, et al. Temporal and spatial dynamics of corticosteroid receptor down-regulation in rat brain following social defeat. *Physiol Behav* 2001;72:349–54.
- [74] Koolhaas JM, Meerlo P, De Boer SF, Strubbe JH, Bohus B. The temporal dynamics of the stress response. *Neurosci Biobehav Rev* 1997;21:775–82.
- [75] Maccari S, Morley-Fletcher S, Mairesse J, Viltart O, Daszuta A, Soumier A, et al. Chronic treatment with agomelatine reversed the decrease in hippocampal cells neurogenesis and survival in prenatally stressed adults rats. *Am Soc Neurosci* 2005;566:8.
- [76] Kempermann G, Jessberger S, Steiner B, Kronenberg G. Milestones of neuronal development in the adult hippocampus. *Trends Neurosci* 2004;27:447–52.
- [77] Brandt MD, Jessberger S, Steiner B, Kronenberg G, Reuter K, Bick-Sander A, et al. Transient calretinin expression defines early postmitotic step of neuronal differentiation in adult hippocampal neurogenesis of mice. *Mol Cell Neurosci* 2003;24:603–13.
- [78] Plumpe T, Ehninger D, Steiner B, Klempin F, Jessberger S, Brandt M, et al. Variability of doublecortin-associated dendrite maturation in adult hippocampal neurogenesis is independent of the regulation of precursor cell proliferation. *BMC Neurosci* 2006;7:77.
- [79] Encinas JM, Vaahtokari A, Enikolopov G. Fluoxetine targets early progenitor cells in the adult brain. *Proc Natl Acad Sci USA* 2006;103:8233–8.
- [80] Wang JW, David DJ, Monckton JE, Battaglia F, Hen R. Chronic fluoxetine stimulates maturation and synaptic plasticity of adult-born hippocampal granule cells. *J Neurosci* 2008;28:1374–84.
- [81] David DJ, Samuels BA, Rainer Q, Wang JW, Marsteller D, Mendez I, et al. Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. *Neuron* 2009;62:479–93.
- [82] Berridge KC. 'Liking' and 'wanting' food rewards: brain substrates and roles in eating disorders. *Physiol Behav* 2009;97:537–50.
- [83] Berridge KC, Robinson TE, Aldridge JW. Dissecting components of reward: 'liking', 'wanting', and learning. *Curr Opin Pharmacol* 2009;9:65–73.
- [84] Bekris S, Antoniou K, Daskas S, Papadopoulou-Daifoti Z. Behavioural and neurochemical effects induced by chronic mild stress applied to two different rat strains. *Behav Brain Res* 2005;161:45–59.
- [85] Koo JW, Duman RS. IL-1 β is an essential mediator of the antineurogenic and anhedonic effects of stress. *Proc Natl Acad Sci USA* 2008;105:751–6.
- [86] Wu HH, Wang S. Strain differences in the chronic mild stress animal model of depression. *Behav Brain Res* 2010;213:94–102.
- [87] Matthews K, Forbes N, Reid IC. Sucrose consumption as an hedonic measure following chronic unpredictable mild stress. *Physiol Behav* 1995;57:241–8.
- [88] Forbes NF, Stewart CA, Matthews K, Reid IC. Chronic mild stress and sucrose consumption: validity as a model of depression. *Physiol Behav* 1996;60:1481–4.
- [89] Nielsen CK, Arnt J, Sanchez C. Intracranial self-stimulation and sucrose intake differ as hedonic measures following chronic mild stress: interstrain and interindividual differences. *Behav Brain Res* 2000;107:21–33.
- [90] Haidkind R, Eller M, Harro M, Kask A, Rinken A, Orelund L, et al. Effects of partial locus coeruleus denervation and chronic mild stress on behaviour and monoamine neurochemistry in the rat. *Eur Neuropsychopharmacol* 2003;13:19–28.
- [91] Willner P. Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS. *Neuropsychobiology* 2005;52:90–110.
- [92] Borsini F, Podhorna J, Marazziti D. Do animal models of anxiety predict anxiolytic-like effects of antidepressants? *Psychopharmacology (Berl)* 2002;163:121–41.
- [93] Millan MJ, Girardon S, Mullot J, Brocco M, Dekeyne A. Stereospecific blockade of marble-burying behaviour in mice by selective, non-peptidergic neurokinin1 (NK1) receptor antagonists. *Neuropharmacology* 2002;42:677–84.
- [94] Gyertyan I. Analysis of the marble burying response: marbles serve to measure digging rather than evoke burying. *Behav Pharmacol* 1995;6:24–31.
- [95] Farley S, Apazoglou K, Witkin JM, Giros B, Tzavara ET. Antidepressant-like effects of an AMPA receptor potentiator under a chronic mild stress paradigm. *Int J Neuropsychopharmacol* 2010;1–12.
- [96] Papp M, Litwa E, Gruca P, Mocaer E. Anxiolytic-like activity of agomelatine and melatonin in three animal models of anxiety. *Behav Pharmacol* 2006;17:9–18.